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Phenotypic Detection of Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* in Urinary Tract Infections at Soba University Hospital

A thesis submitted for partial fulfilment for the requirement of MSc degree in medical laboratory sciences (Microbiology)

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الآية

قال الله تعالى :

بسم الله الرحمن الرحيم

﴿ اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ

وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)﴾

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إلى من آثروني على أنفسهم

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List of abbreviations

Abbreviation	Term
AK	Amikacin
CAZ	Ceftazidime
CDC	Communicable Disease Centre
CFU	Colony forming unit
CIP	Ciprofloxacin
CLED	Cysteine lactose electrolytes deficient
CLSI	Clinical & Laboratory Standards Institute
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
CTX	Cefotaxime
CTX-M	Cefotaxime beta-lactamases
DDCT	Double Disk Combination Test
DDST	Double Disk Synergy Test
DNA	Deoxyribonucleic acid
E.coli	*Escherichia coli*
ESBLs	Extended-Spectrum β-Lactamases
E-test	Epsilometer test
H_2S	Hydrogen sulphide
ICU	Intensive care units
IPM	Imepenem
KIA	Kligler iron agar
KPC	Klebsiella pneumoniae class A carbapenemases
MBL	Cephalosporinases, metallo-β-lactamases
MIC	Minimum inhalation concentration
NCCLS	National Committee for Clinical Laboratory Standards

Abbreviation	Term
NFA	Non fimbrial adhesions
NOR	Norfloxacine
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
STs	Sequence types
SXT	Co-trimoxazole
TE	Tetracycline
TEM-1	Temoniera beta-lactamases class1
TEST	Tigecycline Evaluation and Surveillance Trial
TZP	Pipercilline/tazopactam
UPEC	Uropathogenic Escherichia coli
UTI	Urinary tract infection

Abstract

This study was designed as a prospective laboratory based study in period from March to August 2018; the study was performed to determine the frequency of extended-spectrum β-lactamases (ESBLs) in uropathogenic *E.coli* isolates at Soba University Hospital, Sudan.

A total of 100 *E.coli* isolates were obtained from Soba University Hospital. At microbiology laboratory of National Health Laboratory (ASTAK) the isolates were purified by streaking on nutrient agar plates. Re-identification was done primarily by cultural characteristic, Gram stain and conventional biochemical tests. Antimicrobial susceptibility test was performed by using Modified Kirby-Bauer method and Double Disc Synergy Test (DDST) to determine ESBL production in *E.coli* isolates.

Among the 100 *E.coli* isolates, 56% were ESBLs-positive strains. The frequency of ESBLs production was significantly higher in females 66.7% (42/63) than males 37.8% (14/37); also significantly higher inpatients 64.6% (51/79) than outpatients 23.8% (5/21); however there were no significant differences among age group.

ESBL producers *E.coli* was significantly more resistant to cefotaxime (100%), ceftriaxone (100%), ceftazidime (98.2%) and norfloxacine (89.3%), while the high resistances of non ESBL-producers isolates were to co-trimixazol (54.5%), norfloxacine (52.3%) and tetracycline (43.2%). However the maximum sensitivity of both ESBL-producers and non-producers isolates were seen for imipenem (100% - 100%), followed by piperacillin/tazobactum (80.4% - 97.7%) and amikacin (42.9% - 88.6%). The results of this study suggest that the frequency of ESBLs among uropathogenic *E.coli* is currently in progress in Sudan, and there for further studies is needed.

مستخلص البحث

هذة دراسة مختبرية اجريت في الفترة من مارس الى اغسطس 2018، هدفت الى تحديد إنزيمات البيتالاكتام الممتدة الطيف عند الإسكريشية القولونية المعزولة من مرضى عدوى الجهاز البولي في مستشفى سوبا التعليمي –السودان.

عدد مائة عينة من الإسكريشية القولونية تم عزلها في مستشفى سوبا الجامعي وتم اعادة التعرف عليها في معمل المختبرات المركزية القومية (إستاك) حيث تم زرعها وتنقيتها با ستخدام اوساط الاغار المغذية و تم التعرف على البكتيريا بواسطة خصائصها الاستزراعية وصبغة غرام ، والاختبارات البايوكيميائية المناسبة ، ثم اجريت اختبارت الحساسية للمضادات الميكروبية باستحدام طريقة كريبي المعدلة ، واختبار التآزر ثنائي القرص لتحديد إنزيمات البيتالاكتام الممتدة الطيف.

توصلت هذة الدراسة الى ان 56% من البكتريا المعزولة كانت منتجة لانزيمات البيتالاكتام الممتدة الطيف ، وان هناك فروق ذات دلالة إحصائية بين معدل انتاجها في كل من الذكور37.8% (37/14) والاناث66.7% (63/42) وكذالك بالنسبة لنوعية العينة المعزولة من المرضى المنومين داخل المستشفى 64.6% (79/51) او من العيادات الخارجية 23.8% (21/5) ، بينما لاتوجد فروق ذات دلالة احصائية في انتاج البيتا لاكتام بالنسبة لتوزيع العمري للعينة .

وجدت الدراسة ان الإسكريشية القولونية المنتجة لانزيمات البتالاكتام الممتدة الطيف اكثر مقاومة للمضادات الميكروبية مثل السيفوتاكسيم (100٪) ، سيفترياكسون (100٪) ، السيفتازيديم (98.2٪) والنورفلوكساسين (89.3٪) بينما الغير منتجة لانزيمات البتالاكتام الممتدة الطيف كانت اكثر مقاومة ل كو-ترايمكسازول54.5٪ ، النورفلوكساسين (52.3٪) والتتراسيكلين (43.2٪) ومع ذالك اظرهت كلتا النوعين من البكتريا المنتجة وغير المنتجة لانزيمات البتالاكتام الممتدة الطيف حساسية عالية لكل من امبنم (100% مقابل 100%) ، تليها بيبراسلين / تازوباكتوم (80.4% مقابل 97.7%) و اميكاسين (42.9% مقابل 88.6%). اظهرت نتائج هذة الدراسة ان معدل حدوث اصابات عدوى الجهاز البولي ببكتريا الإسكريشية القولونية المنتجة للانزيمات البتالاكتام الممتدة الطيف في زيادة مستمرة في السودان مما يستدعي اجراء مزيدا من الدراسات المتطورة و الحديثة .

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1.1. Introduction

Beta-lactam (β-lactam) antibiotics are wide range group of antibiotics that are commonly used to treat infectious diseases (CDC, 2009). They consist of the antibiotic agents that enclose in their molecular structure a β- lactam ring. Many examples may be encountered of such as carbapenems, monobactams, cephalosporins and penicillins (Lee *et al*., 2009). Cephalosporins antibiotics are known for its broad spectrum activity, proven efficacy and favorable safety profile, making it the most commonly prescribed class of antimicrobials (Laudano, 2011). Many strains of bacteria developed resistance against β-lactam antibiotics via production of β-lactamase which are an enzyme that break down the β-lactam ring (Lee *et al*., 2009). Resistance in gram-negative bacteria is increasing; this is mainly due to the spread of strains producing Extended-Spectrum β-Lactamases (ESBLs). Many of the isolates producing these enzymes are also resistant to trimethoprim, quinolones and aminoglycosides, often plasmid has co-expression of other resistance mechanisms (Pallett and Hand, 2010). Extended-Spectrum β-Lactamases (ESBLs) are rapidly evolving groups of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam, yet are inhibited by Clavulanic acid (Paterson and Bonomo, 2005). ESBL-producing bacteria are associated with severe infections such as bacteremia, intra-abdominal infection, urinary tract infections, and respiratory tract infections (Dhillon and Clark, 2012). Options in the treatment of ESBL-producing organism infections are extremely limited; carbapenems are the treatment of choice for serious infections due to such organisms. The presence of ESBLs carries tremendous clinical significance (Paterson and Bonomo, 2005). To overcome this resistance, scientists developed a new class of antibiotics. Which are β- lactamase inhibitors, for

example clavamox which is combination of amoxicillin and clavulanic acid (Bratu *et al*., 2005).

Extended-spectrum beta-lactamase (ESBL)-producing *E.coli* is an increasingly important group of community pathogens worldwide, these organisms are frequently resistant to many of the antimicrobial agents usually recommended for the treatment of infections caused by *E. coli*, such as penicillin's, cephalosporin's, fluoroquinolones, and trimethoprim-sulfamethoxazole, data concerning risk factors, clinical features, and therapeutic options for such infections are scarce (Rodríguez-Baño *et al*., 2008). Extended-spectrum beta-lactamase (ESBLs) are enzymes produced by *E.coli* , *Klebsiella* and other pathogens, these enzymes proved resistance to many of the antimicrobial agents usually recommended for the treatment of urinary tract infections such as penicillin, sulfamethoxazole, trimethoprim and cephalothin were the resistant is 100%, 30.89%, 16.26% and 20.32%, respectively (Momtaz *et al*., 2013).

Urinary tract infection (UTI) is known to affect approximately 150 million people each year. It is the second most common infection, and is responsible for approximately seven million doctor visits per year. The frequency of infection varies especially with age and sex. Among uropathogens, *Escherichia coli* is responsible for 80% of community-acquired UTI and 40% of healthcare-associated UTI. Other uropathogens include *Candida spp*, *Proteus mirabilis*, *Staphylococcus* and *Klebsiella spp* (Khan *et al*., 2013) .

1.2. Rationale

Enterobacteriaceae have become one of the most important causes of nosocomial and community-acquired infections. Recently, many articles reported increased incidence of urinary tract infection (UTI) due to Extended-Spectrum Beta Lactamase (ESBL) – producing E. coli. Therefore the present study aimed to detect ESBL positive and multidrug resistant strains among UTIs caused by *E. coli* isolates against commonly used antimicrobial agents.

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1.3 Objectives

1.3.1. General Objective:

To detect the extended-spectrum beta-lactamase producing *Escherichia coli* among urinary tract patients in Soba University hospitals.

1.3.2. Specific Objectives:

- To re-identify *E.coli* among UTIs patients.
- To detect ESBL producer *E.coli* among UTIs patients.
- To determine the distribution of ESBLs producing *E.coli* isolates according to patients age, sex, inpatient department and outpatient department.
- To assess and compare the antimicrobial resistance profile among the extended-spectrum beta-lactamase producing *E.coli* and non-producing *E.coli*.

Chapter Two

Literature Review

Literature Review

2.1 Antibiotics.

Most antibiotics probably evolved millions of years ago as the result of competition for survival between different microorganisms in soil, plants, and the oceans. Thus, these substances most likely represent part of the evolution and the competition that allows a species to dominate within an ecological niche (Alsterlund *et al.*, 2009). In 1909, Paul Erlich and colleagues developed the first synthetic antibacterial compound, arsphenamine (Salvarsan), but it had many adverse effects. The first commercially available antibiotic was sulfonamide (Prontosil), which was discovered by Gerhard Domagk in 1932. Also, as early as 1928, Alexander Fleming found that the fungus *Penicillium* had an antibacterial effect, but it was not until Howard Florey and Ernst in Chain developed penicillin in 1940 and after World War II that the first ß-lactam antibiotic became available on the market. During the1950s and 1960s, a massive investigation of soil samples from all over the world was launched to identify active compounds. *Actinomycetes*`(especially subspecies of the genus *Streptomyces*) were found to be some of the most valuable microorganisms for producing antibiotic agents, and a typical pharmaceutical company at that time performed research on as many as 100000 different *Actinomycete*s in single a year (Tumbarello *et al.*, 2006). In 1943, Albert Schatz discovered the first aminoglycoside, streptomycin, which also proved to be the first anti-infective agent that could provide protection against tuberculosis. The polymyxines were detected in and derived from soil bacteria in 1947, and erythromycin was discovered in soil samples from the Philippines in 1949. Azithromycin, clarithromycin, and the ketolides were obtained through further development of erythromycin. Also, nitro groups were introduced into furans that had been used in the 1940s, and this led to nitrofurantoin, which was put on the market in the 1950s. In the late 1940s, Benjamin Minge Duggar

discovered chlortetracycline, and Burkholder and colleagues found chloramphenicol in one out of7000 samples collected in Caracas, Venezuela. In the mid-1950s, vancomycin was isolated from an organism found in soil samples in Borneo, and it was introduced on the market in 1958. Rifamycin was discovered in 1957 and named after a French movie (Rififi), and metronidazole was presented in 1959 (Tumbarello *et al.*, 2006). The sodium salt of fusidic acid (brand name Fucidin) was developed by Godtfredsen at Leo Laboratories in Denmark, and it was introduced in clinical practice in 1962. The same year, lincomycin was found in a soil organism in Nebraska in the United States, and Lesher identified the first quinolone nalidixic acid among the by-products of chloroquine. In the late 1960s, Bushby and Hitchings synthesized a sulfonamide potentiator called trimethoprim, and, when it was combined with sulpha, its antibiotic effect became bactericidal co-trimoxazole. In 1969, Hendlin *et al*. discovered a new cell-wall-active antibiotic produced by several *Streptomyces* species, and this agent was first called phosphonmycin but later renamed fosfomycin. Walter Gregory and co-workers at Dupont synthesized oxazolidin ones that were registered in 1978, but it took an additional 25 years of investigation before they had a useful drug on the market. Since then, only a few classes of antibiotics have become commercially available, among them the glycylcycline (tetracycline analogue) tigecycline, which was introduced in 2005 and launched the same year, and in 2012, the microcyclic antibiotic fidaxomicin, which was obtained from *Actinomycetes*. Fidaxomicin has a bactericidal effect on *Clostridium difficile* infections. The discovery of antibiotics is considered to be one of the most valuable findings related to human health (Stansly *et al.*, 1949).

2.2. β –lactamases.

The β-lactamases are the collective name of enzymes that open the ß-lactam ring by adding a water molecule to the common ß-lactam bond, and this inactivates the

ß-lactam antibiotic from penicillin to carbapenems. This hydrolyzation was first observed in 1940 by Abraham and Chain (penicillinase) in a strain of *E. coli* (Jacoby, 2009). β- Lactamases are hydrolytic enzymes with the ability to inactivate β-Lactam antibiotics before they reach the penicillin-binding proteins located at the cytoplasmic membrane (Falagas and Karageorgopoulos, 2009; Tham, 2012). Many of the gram negative bacteria possess a naturally occurring chromosomally mediated β-lactamase, which probably assists the bacteria in finding a niche when faced with competition from other bacteria that naturally produceβ-lactams (Turner, 2005; Tham, 2012). The first plasmid-mediated β-lactamase in Gram negative bacteria, *TEM-1*, was described in 1965. This occurred in a strain of Escherichia coli isolated from blood culture of a patient in Greece ("*TEM*" came from the patient's name, Temoniera).Because this β-lactamase was plasmid - borne, has been spread to other members of the Enterobacteriaceae family, *H. influenzae, Neisseria gonorrhoeae* and *Pseudomonas aeruginosa.* Another plasmid mediated β-lactamase, known as"*SHV-1*" (sulfhydryl variable), was found In *Klebsiella pneumonia* and *E. coli* (Turner, 2005). The presence of these enzymes influenced the efforts of pharmaceutical companies' to negate their effects. One such development was that of the oxyimino-cephalosporins (third generation of cephalosporin's), which showed good stability against the *TEM-1* and *SHV-1* β-lactamases. This class of antibiotics was widely used for the treatment of serious hospital infections due to gram negative organisms (Turner, 2005).

2.3. β-lactam antibiotics.

The bactericidal effect of ß-lactam antibiotics involves inhibition of cell wall synthesis, and this effect occurs through covalent attachment to penicillin-binding protein (PBP), which is a peptidoglycan trans-peptidase enzyme that catalyzes the final steps in cell wall formation. Damage of the bacterial cell by hydroxyl radicals also plays a role in this process, but the exact mechanism is still somewhat unclear.

Several PBPs have been identified, and they are unique to bacteria. Furthermore, the spectrum and effects of the different ß-lactams are determined by the PBPs to which these antibiotics bind (Jacoby, 2009). The first successful clinical treatment with penicillin was achieved in 1930 by Cecil George Paine at the Sheffield Royal Infirmary, when he used Fleming droplets to treat gonococcal ophthalmia neonatorum (conjunctivitis in newborns). Paine did not publish his results, but many years later (i.e., in 1983) when his discovery was made public, he said "I was a poor fool who didn't see the obvious when placed in front of me". American companies started to produce penicillin G, whereas the British produced penicillin F. In Austria, Brandl and Margreiter found the more acid-stable penicillin V, which represented the first active penicillin for oral administration. Ampicillin and amoxicillin (α -aminopenicillins), two penicillin derivates with greater acid stability and a better Gram-negative effect, were developed by Beecham (Jacoby, 2009).

2.4. Extended Spectrum β –Lactamases (ESBLs).

Extended-Spectrum β-Lactamases (ESBLs) are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam, yet are inhibited by clavulanic acid. Typically, they derive from genes of *TEM-1*, *TEM-2*, or *SHV-1* by mutations that alter the amino acid configuration around the active site of these β-lactamases. The first report of plasmid-encoded β-lactamases capable of hydrolyzing the extended-spectrum cephalosporins (*SHV-2*) was published in Germany, 1983 (Paterson and Bonomo, 2005). These enzymes can be carried on bacterial chromosomes, that is, inherent to the organism, or may be plasmid-mediated with the potential to move between bacterial populations. ESBLs are primarily produced by the Enterobacteriaceae family, in particular *Klebsiella pneumonia* and *Escherichia coli*. They are also

produced by non -fermentative Gram negative organisms, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Dhillon and Clark, 2012).

2.4.1. ESBLs classification.

The total number of ESBLs now exceeds 200 enzymes.β-Lactamases are most commonly classified according to two general schemes: the Ambler molecular classification scheme and the Bush-Jacoby-Medieros classification scheme. The Ambler scheme divides β-lactamases into four major classes (A to D) according to protein homology (ESBLs are in class A)(Paterson and Bonomo, 2005;Dhillon and Clark, 2012). The Bush-Jacoby-Medeiros classification scheme groups β-lactamases according to functional similarities (substrate and inhibitor profile). ESBLs classified in Bush-Jacoby-Medieros functional classification as 2be β – lactamases. 2be designation shows that these enzymes are derived from group 2b β-lactamases (for example, *TEM-1*, *TEM-2*, and *SHV-1*); the "e" of 2be denotes that the β-lactamases have an extended spectrum (Paterson and Bonomo, 2005).

There are various genotypes of ESBLs; the most common are the *SHV*, *TEM*, and *CTX-M* types. Other clinically important types include *VEB*, *PER*, *BEL-1*, *BES-1*, *SFO-1*, *TLA*, and *IBC* (Dhillon and Clark, 2012). These classifications exclude all other β- lactamases such as plasmid-borne *AmpC* or *OXA*-type Cephalosporinases, metallo-β-lactamases (*MBL*),*OXA* type Carbapenemases, the *Klebsiella pneumonia* class A carbapenemases (*KPC*) and certain *GES*-variant β-lactamases which have different functional and/or structural classes, but they all certainly share an extended spectrum of β-lactam hydrolysis(Giske *et al*., 2009).

Giske *et al*., (2009) propose that the classical, functional class 2be β- lactamases could be designated as 'class A ESBLs' (ESBLA), whereas plasmid-mediated *AmpC* and *OXA*-ESBLs could be labeled 'miscellaneous ESBLs' (ESBLM) and ESBLs with hydrolytic activity against Carbapenems' (ESBLCARBA).

2.4.2. The global epidemiology of ESBLs.

The epidemiology of ESBLs is quite complex. First, there are several different levels to consider: the wider geographical area, the country, the hospital, the community, and the host (in most cases a single patient or a healthy carrier). Furthermore, there are the bacteria (*E. coli are* more endemic) and their mobile genetic elements, usually plasmids. In addition, there are numerous reservoirs, including the environment (e.g., soil and water), wild animals, farm animals, and pets. The final component entails transmission from food and water, and via direct or indirect contact (person to person) (Briongos *et al.*, 2012).The specific uropathogenic *E. coli* clone ST131, which has been associated with carriage of the ESBL *CTX-M-15* and quinolone resistance, has probably contributed to the successful spread of the ESBL-expressing bacteria around the world (Briongos *et al.*,2012) When ESBLs were first recognized in the early 1980s, they have become a major cause of hospital-acquired infection, particularly in the intensive care units (ICU).*TEM* and *SHV-types* have been recognized across the world with over 100 mutations (Dhillon and Clark, 2012). The *CTX-M* enzymes appear to have a greater ability to spread and cause outbreaks. There are over 50 variants of *CTX-M* to date, and they have been associated with numerous outbreaks of infections both in hospitals and in the community (Dhillon and Clark, 2012).

Data from the last 10 years establishes *CTX-M* genotype as the predominant ESBL in Europe and East Asia. The prevalence of bacteria producing ESBLs varies worldwide, with reports from North America, South America, Europe, Africa, and Asia. Data from the Tigecycline Evaluation and Surveillance Trial (TEST) global surveillance database shows the rate of ESBL production was highest among the *K.pneumoniae* isolates collected in Latin America, followed by Asia/Pacific Rim, Europe, and North America (44.0%, 22.4%, 13.3%, and7.5%, respectively) (Dhillon and Clark, 2012).). Since 2000, the European Antimicrobial Resistance

Surveillance Network has reported a steady increase in the rates of invasive *E. coli* and *Klebsiella pneumonia* isolates resistant to third-generation and fourth generation cephalosporins. ESBLs confer resistance to oxyimino-cephalosporins, and often express a multidrug-resistant phenotype, leaving only limited therapeutic options (Ewers *et al*., 2012). In comparison with the rest of the world, there is generally a lack of comprehensive data regarding ESBL producing Enterobacteriaceae in African countries. However, there is sufficient evidence to highlight the prevalence of ESBLs in Africa. It is recognized that Egypt has an extremely high rate of ESBL producers, with up to 70% of isolates producing the enzyme. The *CTX-M* genotype appears to be the most common type in North Africa. There have also been reports of *CTX-M K. pneumoniae* in Kenya and *SHV* and *TEM*-types in South Africa (Dhillon and Clark, 2012).

2.5. Phenotypic detection of ESBL enzymes.

2.5.1. Disk-Diffusion method.

Screening test with an indicator cephalosporin's which looks for resistance or diminished susceptibility, thus identifying isolates likely to be harboring ESBLs. The Clinical and Laboratory Standards Institute (CLSI) has proposed disk-diffusion methods for screening for ESBL production by *Klebsiellae pneumoniae, K. oxytoca, Escherichia coli* and *Proteus mirabilis*. Disk-diffusion methods were used for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. Cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone disks are used. Since the affinity of ESBLs for different substrates is variable, the use of more than one of these agents for screening improves the sensitivity of detection. However, it is adequate to use cefotaxime, which is consistently susceptible to *CTX-M*; and ceftazidime, which is a consistently good substrate for *TEM* and *SHV* variants. If isolates show resistance or diminished susceptibility to any of these

agents, it indicates suspicion for ESBL production, and phenotypic confirmatory tests should be used (Rawat and Nair, 2010).

2.5.2. Dilution antimicrobial susceptibility tests.

The CLSI has proposed dilution methods for screening for ESBL production by *Klebsiellae pneumoniae*, *K oxytoca*, *Escherichia coli* and *Proteus mirabilis*. Ceftazidime, aztreonam, cefotaxime or ceftriaxone can be used at a screening concentration of 1 μg/mL or cefpodoxime at a concentration of 1 μg/mL for *Proteus mirabilis*; or 4 μg/mL, for the others. Growth at or above this screening antibiotic concentration is suspicious of ESBL production and is an indication for the organism to be tested by a phenotypic confirmatory test (Rawat and Nair, 2010).

2.5.3. Double Disk Combination Test (DDCT).

The British Society for Antimicrobial Chemotherapy has recommended the disk-diffusion method for phenotypic confirmation of ESBL presence using ceftazidime/clavulanate and cefotaxime/clavulanate combination disks. Using this method, the zone diameters of each combination compared with those of the cephalosporin alone, and calculated a ratio of cephalosporin/clavulanate zone size divided by cephalosporin zone size. A ratio of 1.5 or greater was taken to signify the presence of ESBL activity (Rawat and Nair, 2010).

2.5.4. Double Disk Synergy Test (DDST).

In this, test disks of third-generation cephalosporins and amoxicillin/
clavulanate (Augmentin) are kept 30 mm apart, center to center, on inoculated Mueller-Hinton agar. A clear extension of the edge of the inhibition zone of cephalosporin towards Augmentin disk is interpreted as positive for ESBL production. Evaluations of the double-disk diffusion test have revealed sensitivities of the method ranging from 79% to 97% and specificities ranging from 94% to 100%. In isolates which are suspicious for harboring ESBLs but are negative using

the standard distance of 30 mm between disks, the test should be repeated using closer (for example, 20 mm) or more distant (for example, 40 mm) spacing (Rawat and Nair, 2010).

2.5.5. E-test for ESBLs.

plastic drug-impregnated strips, one end of which contains a gradient of ceftazidime (MIC test range 0.5 to 32 μg/ml) and the other with a gradient of ceftazidime plus a constant concentration of clavulanate (4 μg/ml). Similar strips containing cefotaxime and cefotaxime/clavulanate. These strips are useful for both screening and phenotypic confirmation of ESBL production. The reported sensitivity of the method as a phenotypic confirmatory test for ESBLs is 87 to 100% and the specificity is 95 to 100%. The sensitivity and specificity of the method depend on the ratio of MICs of the cephalosporin versus cephalosporin/clavulanate combination used (Rawat and Nair, 2010).

2.6. ESBL infections and treatment.

ESBL-producing organisms have an enormous clinical and microbiological significance. Such bacteria are associated with severe infections such as bacteremia, intra-abdominal infection, urinary tract infections and respiratory tract infections. They inactivate cephalosporins, which are often used in treating the septic patient in a variety of clinical settings. Therefore, this often renders empiric antibiotic treatment ineffective. Many ESBL genes have the propensity to jump between organisms, thus leading to outbreaks of infection, if this occurs in an easily transmissible pathogen. It is also known that organisms producing ESBLs also have the ready capacity to acquire resistance to other antimicrobial classes such as the quinolones, tetracyclines, cotrimoxazole, trimethoprim, and aminoglycosides, which further limits therapeutic options (Dhillon and Clark, 2012). Nosocomial infections caused by these organisms complicate therapy and limit treatment options, in addition, patients infected with ESBL-producing

bacteria may have a higher mortality rate and may require longer hospital stays because they are generally sicker and have received more antibiotics than patients who are not infected with ESBL-producing strains (Ramphal and Ambrose, 2006). Effective strategies for the empirical and directed treatment of infections caused by ESBL-producing pathogens include the use of carbapenems and, possibly, the fourth-generation cephalosporin cefepime. Studies indicate that the use of cefepime to treat serious nosocomial infections (e.g., bacteremia, pneumonia, and urinary tract infections) is associated with high rates of microbiological and clinical success (Ramphal and Ambrose, 2006). The choice of a Sulfonamide or Sulfonamide-containing combination, Penicillin, Cephalosporin or Nitrofurantoin, based on the results of susceptibility testing, are appropriate regimens for the management of asymptomatic bacteriuria. Increasing antibiotic resistance, however, complicates the choice of empiric regimens and is likely to become an increasing problem (Smaill and Vazquez, 2007).

2.7. Prevention and control of ESBLs.

- Consistent use of Routine Practices with all patients/ residents/ clients
- Initiate Contact Precautions for patients/residents with an ESBL infection Appropriate client/patient/resident placement
- Gloves for all activities in the patient's room or bed space in acute care, or for direct care of clients/residents in long-term care and ambulatory/clinic settings
- Long-sleeved gown for activities where skin or clothing will come in contact with the patient or their environment in acute care, or for direct care of clients/residents in long-term care and ambulatory/ clinic settings
- Dedicated equipment or adequate cleaning and disinfecting of shared equipment, with particular attention to management of urinary catheters and associated equipment.

- Notify the Infection Prevention and Control Practitioner or delegate to discuss the infection control management of client/ patient/ resident activities
- Precautions are not to be discontinued until reviewed by Infection Prevention and Control (Donnenberg, 2009).

2.8. Resistance in Gram negative bacteria.

Gram negative bacteria are organisms acquiring genes that code for mechanisms of antibiotic drug resistance, especially in the presence of antibiotic selection pressure. They often using multiple mechanisms against the same antibiotic or using a single mechanism to affect multiple antibiotics (Peleg and Hooper, 2010). Seven mechanisms of resistance can be used by Gram negative bacteria, with some being mediated by a mobile plasmid. These mechanisms include the loss of porins, which reduces the movement of drug through the cell membrane; the presence of β-Lactamases in the periplasmic space, which degrades the β-lactam; increased expression of the trans-membrane efflux pump, which expels the drug from the bacterium before it can have an effect; the presence of antibiotic-modifying enzymes, which make the antibiotic incapable of interacting with its target; target site mutations, which prevent the antibiotic from binding to its site of action; ribosomal mutations or modifications, which prevent the antibiotic from binding and inhibiting protein synthesis; metabolic bypass mechanisms, which use an alternative resistant enzyme to bypass the inhibitory effect of the antibiotic; and a mutation in the lipopolysaccharide, which renders the polymyxin class of antibiotics unable to bind this target (Peleg and Hooper, 2010). β- Lactamases are the primary mechanism of conferring bacterial resistance to β-Lactam antibiotics, such as Penicillins and Cephalosporins (Dhillon and Clark, 2012).

Enterobacteriaceae expressing Extended Spectrum β-Lactamase (ESBL) are among the most multidrug-resistant pathogens in hospital and spreading worldwide. Transient carriage of bacteria on hands of health care workers may lead

to transmission to patients (Tschudin-sutter *et al*.,2010). A report done by Tschudin-sutter *et al*., (2010) revealed that an outbreak caused by transmission of ESBL *E.coli* from a mother to her new born twins and subsequent spread to other neonates and one health care worker. The mother was most colonized before hospitalization and UTI developed peripartum. Transmission by contact during vaginal delivery of twins and transmission by physical contact to health care worker and other neonates was the most likely mode of transmission.

2.9. *Escherichia coli.*

Escherichia coli is the most prevalent facultative anaerobic species in the human gastrointestinal tract (109 CFU/g faeces) but it also colonizes the intestines of animals and is thus used as an indicator of faecal contamination of drinking water and food. E.coli is usually a harmless microbe, although it is also the most common cause of community acquired bacteraemia and the fifth most common cause of nosocomial bacteraemia (Rasko *et al*., 2008). The more virulent pathotypes often have a larger genome compared to the non-pathogenic *E. coli*, and there are also many different virulence factors, which are usually encoded on plasmids, chromosomes, or bacteriophages (Nicolas, 2008). The serotypes and groups of pathogenic *E. coli* are defined by their lipopolysaccharide (O) and flagellar (H) antigens. Geographically widespread epidemic clones with the same chromosomal sequence types (STs) have been identified among *E. coli* strains that cause urinary tract infections. Extended-spectrum beta-lactamase (ESBL)-producing strains are usually community acquired, and only a few hospital outbreaks of such bacteria have been reported (Alsterlund *et al*., 2012).

2.10. Urinary tract infection.

Urinary tract infection (UTI) is a general term referring to the infection anywhere in the urinary tract. This is among the most common serious bacterial infections in infants and children (Wald, 2004). UTI is a common cause of morbidity in children

both in community and hospitalized patients (Peterson, 2004). If UTI is not diagnosed early and treated adequately, it may result into chronic illness and long term renal damage (Adjei and Opoku, 2004). Such an infection manifests either solely in the lower urinary tract (urethritis, cystitis, urethrocystitis) or affects the renal pelvis and kidneys (cystopyelitis, pyelonephritis). In acute urinary tract infections, *E. coli* is the causative organism in 70–80% of cases and in chronic, persistent infections in 40–50% of cases. Urinary tract infections result from ascension of the pathogen from the ostium urethrae. Development of such an infection is also furthered by obstructive anomalies, a neurogenic bladder or a vesicoureteral reflux. Urinary tract infections that occur in the absence of any physical anomalies are often caused by the path ovar UPEC (uropathogenic *E. coli*). UPEC strains can attach specifically to receptors of the renal pelvis mucosa with pyelonephritis-associated pili or non fimbrial adhesions (NFA). They produce the hemolysin HlyA.

2.11. Previous studies.

- In study done by Ahmed *et al*. (2013) under title – Increasing prevalence of ESBL-producing *Enterobacteriaceae* in Sudan community patients with UTIs, found ESBL producing *E. coli was* 65.0 %. ESBL producing *E. coli showed* maximum resistance to Ceftazidime 95.4%, followed by Cefotaxime 94.6%, while minimum resistance was seen with Impenem 0%.
- Prevalence of Extended-Spectrum β-Lactamases-producing *E. coli* from Hospitals in Khartoum State, Sudan a study done by Ibrahim *et al*. (2013) aimed to determine the prevalence and assess antimicrobial susceptibility of Extended- Spectrum β-Lactamases-producing *Escherichia coli* isolated from clinical specimens of patients at hospitals in Khartoum State, Sudan; showed that (30.2%) *E. coli isolates* were found positive for ESBL by the applied phenotypic methods.

- Mekki *et al*. (2010) in another Sudanese study carried out in Khartoum state hospitals to evaluate emergence of ESBL among multidrug- resistant *Escherichia coli* and *Klebsiella* species causing nosocomial UTI,β- Lactamase was produced by all isolates; high resistance level for third generation Cephalosporin was noticed. ESBLs were detected in high prevalence among all multidrug -resistant *E. coli and Klebsiella* species isolates 53%.
- In Saudi Arabia Hassan et al. (2014) detect ESBL production in *E. coli* 35.8% and *K. pneumonia* 25.7% out of 382 *Enterobacteriaceae* clinical s samples were processed for culture and antimicrobial sensitivity testing.
- In Nigeria ESBL was detected in 47.1% of the 85 isolates and *E. coli was* the major ESBL producer 52.5% followed by *K. pneumoniae* 47.5%. This study done by Azekhueme *et al*. (2015) aimed to investigate the prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in clinical Amples and determine their antimicrobial susceptibility profile.
- In (2006) a study done by Yu *et al*. in Taiwan showed the ESBLs producer *E. coli* were 1.5-16.7% and ESBLs producer *K.pneumoniae* were 8.5-29.8%.
- Indian study done by Sharma *et al*. published in (2013) showed that ESBLs producer positive in 57.2% and *K. pneumoniae* producing ESBL were 67.04%.

Chapter Three

Materials and Methods

Materials and Methods

3.1. Study design:

This is a prospective laboratory-based study.

3.2. Study area:

The study was carried out on the isolates of *E. coli obtained* from the microbiology laboratory of Soba University Hospital, which isolated from patients with urinary tract infection, and the investigation work was carried out in microbiology laboratory of National Health Laboratory (ASTAK) Sudan.

3.3. Sample size:

100 *E. coli* isolates from urinary tract infected patients.

3.4. Study duration:

The study was conducted during the period from March to August 2018.

3.5. Data collection tools:

The data was collected from the investigation request lab form of Soba University Hospital.

3.6. Re-identification of the isolates.

3.3.1. Checking purity.

The isolates were cultured on nutrient agar and incubated at 37^{o}C for 18-24 hrs. Purity of each isolate was checked microscopically following simple stain as described by Cheesbrough, (2006).

3.6.2. Gram Stain.

Gram stain was essential technique for initial identification of bacterial isolates. The procedure was carried out according to Cheesbrough (2006) as follows; smear was prepared from overnight culture on a clean and dry slide. The smear was left to air dry. Fixation was done by rapid pass of the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30–60 seconds, and then washed by tap water. Lugol's

iodine was added for 30-60 seconds then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. Finally, the smear was covered with saffranin stain for 2 minutes and washed by tap water. The back of slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X.

3.6.3. Biochemical tests.

Sets of biochemical tests were used for re-identification of E.coli, Including (Oxidase test, KIA medium, Citrate utilization test, Urease test, Indole test, motility test).

3.6.3.1. Oxidase test.

This test was used to detect bacteria which have ability to secrete cytochrome oxidase enzyme which react with oxidase reagent (tetramethyl para-phenylene diamine dihydrochloride) to give deep purple color. Under aseptic conditions tested bacteria were smeared on disc impregnated in oxidase reagent, and it immediately no change in color of discs, the results was reported as oxidase negative. In oxidase positive discs colour change into deep purple color. (Cheesbrough, 2006).

3.6.3.2. Kligler iron agar (KIA).

KIA media was used for identification of bacteria having the ability to ferment lactose with or without gas and hydrogen sulfide (H_2S) production. Tested bacteria were inoculated in KIA media (HiMedia, India) under aseptic conditions and incubated overnight at 37˚C. At end of the incubation period, color, gas and H_2S were observed. Fermenting Lactose is producing acid which convert the pH of media to acidic pH which in presence of phenol red (indicator) change colour of medium from red to yellow gas detected by air bubbles and cracking and hydrogen sulfide (H_2S) by blacking the media (Cheesbrough, 2006).

3.6.3.3. Citrate utilization test.

This test was used to identify bacteria which have ability to utilize sodium citrate as sole source of carbon. After inoculation the tested bacteria in Simmons citrate agar (HiMedia, India), incubated overnight at 37˚C. The colour of media was observed at end of incubation period and the results were reported. Bromothymole blue (indicator) is green in neutral pH and converted to blue colour due to presence of sodium carbonate which is alkaline compound (Cheesbrough, 2006).

3.6.3.4. Urease test.

Urease test was used to detect bacteria which have ability to secrete urease enzyme. Under aseptic conditions Christensen media (HiMedia, India) were inoculated with tested bacteria and incubated for overnight at 37˚C and at the end of incubation period the results were reported. This enzyme cans breakdown urea in to ammonia and carbon dioxide. Ammonia converts pH of media to alkaline which change the colour of the Christensen medium from colorless to magenta or pink color due to presence of phenol red as indicator, which consider as positive test (Cheesbrough, 2006).

3.6.3.5. Indole test.

This test was used to detect bacteria which have ability to produce indole after breakdown of the amino acid tryptophan. Tested bacteria were inoculated in peptone water which contains tryptophan (HiMedia, India) and incubated for overnight at 37˚C. Indole production was detected by adding drops of Kovac's reagent (HiMedia, India). When red ring appear in seconds, tested organism was reported as positive result (Cheesbrough, 2006).

3.6.3.6. Motility test medium.

This test was used to detect motile bacteria in semi-solid media. After inoculation the tested bacteria by stabbing the semi-solid media with straight wire, incubated overnight at 37˚C. The motility of bacteria was detected by turbidity around stabbed area at end of incubation period and the results were reported.

3.7. Susceptibility test.

A modified Kirby- Bauer susceptibility testing method was used to assess the sensitivity and resistance patterns of the isolates. On Mueller Hinton agar (HiMedia, India), a suspension of tested isolate which was compared with 0.5 % Macfarland standard was seeded. A set of antibiotics discs were applied include imepenem10µg, ciprofloxacin 30µg, co-trimoxazole 30µg, ceftazidime 30µg, cefotaxime 30µg, ceftriaxone 30µg, amikacin (30 µg), norfloxacine, tetracycline and pipercilline/tazopactam (HiMedia, India). Plates were incubated aerobically for overnight at 37˚C. Zones of inhibition were measured in mm and compared to a standard interpretation chart (Cheesbrough, 2006).

3.8. Double Disc Synergy Test (DDST).

This test was used to detect Extended Spectrum β-Lactamases (ESBLs). All E.coli isolates which showed a diameter of or less than 17 mm for Ceftazidime and of or less than 22 mm for Cefotaxime were selected for checking the ESBLs production, the production of ESBL was tested by using a disc of Amoxicillin/Clavulanic acid (20/10µgHiMedia, India) along with two third generation Cephalosporins; Ceftazidime (30µg) and Cefotaxime (30µg) discs, on Mueller Hinton agar plates lawn of tested strains and *E.coli* ATCC 25922 (negative control) were made. Amoxicillin/Clavulanic acid (20/10µg) disc was placed in the center of the plate and Ceftazidime (30µg) and Cefotaxime (30µg) discs were placed 15 mm apart center to center to Amoxicillin /Clavulanic acid and incubated for 18-24 hours at

37˚C. Any increase in the zone towards the disc of Amoxicillin /Clavulanic acid was considered as positive result for the ESBL production (Kaur *et al*., 2013).

3.3.4.2. Quality control.

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *E. coli* ATCC 25922 as ESBL negative and anther *E. coli* strain known as ESBLs positive by phenotypic and genotypic method (PCR and DNA sequencing) was used as a positive control obtained from the Central Public Health Laboratory.

3.4 Ethical consideration.

Approval was taken from Shendi University and from Soba University Hospital.

Chapter Four

Results

Results

A total of 100 *E. coli isolates* were obtained from Microbiology Laboratory of Soba University Hospital, Sudan. The isolates were re-identified in microbiology laboratory of National Health Laboratory (ASTAK) by using gram stain and biochemical test.

The isolates were obtained from both inpatients 79 (79%) and outpatients 21 (21%) and the distribution of ESBL producing *E. coli* inpatient*s* were 48(85.7%) and outpatients were 8(14.3%) (Table 4.3). In this study, ESBL producing *E. coli* were 56%, the non- ESBL producers *E. coli* were 44% (Table 4.1). The isolates were recovered from both females 63 (63%) and males 37 (37%) and the distribution of ESBL producing *E. coli* among females were 42(75%) and males were 14(25%) (Table 4.2). The distribution of ESBL producer *E. coli* according the age divided into three groups which the higher ESBL producer *E. coli* were 21 (53.8%) in ≥ 46 years age groups followed by 20 (62.5%) in ≤ 16 years age groups and 14 (48.3%) in 17-45 years age groups (Table 4.3).. Antibiotic susceptibility pattern of *E. coli* and antibacterial resistance of ESBL and non-ESBL producing *E. coli* among urinary isolates are shown in (Table 4.5). The antibiotic sensitivity pattern of the *E. coli* isolates revealed that the maximum sensitivity was seen for imipenem (100%), followed by piperacillin/tazobactum (88%), amikacin (63%). ESBL phenotype producing *E. coli* showed maximum resistance to cefotaxime (100%) and ceftriaxone (100%), followed by ceftazidime (98.2%) while minimum resistance was seen with imipenem (0%) and piperacillin/tazobactam (12.5%).The non-ESBL phenotype producing *E. coli* showed maximum resistance to Co-trimixazol (54.5%) followed by norfloxacine (52.3%), both co-amoxiclav and tetracycline were (43.2%), ceftazidime (38.6%) while the minimum resistance was

seen with imipenem (0%),followed by piperacillin/tazobactam (2.3%) ,amikacin (11.4%) and ceftriaxone (27.3%).

Table (4.1): Shows the distribution of ESBLs productions among uropathogenic *E. coli.*

E. coli / ESBL	NO	Percentage
Producer	56	56
Non-producer	44	44
Total	100	100

Table (4.2): Shows the distribution of ESBLs producer and non-ESBLS producer *E. coli* isolates with association to gender.

Gender	ESBLs		Total	P. value
	Positive NO - (%)	Negative NO - (%)	NO - (%)	
Male	14 (37.8%)	23 (62.2%)	37 (37%)	0.005
Female	42 (66.7%)	21 (33.3%)	63 (63%)	
Total	56 (56%)	44 (44%)	100 (100%)	

Table (4.3): Shows the distribution of ESBLs producer and non-ESBLs producer *E. coli* isolates with association to age.

Age group	ESBLs		Total NO - (%)	P. value
	Positive NO - (%)	Negative NO - (%)		
≤ 16 years	20 (62.5%)	12 (37.5%)	32 (32%)	0.372
17-45 years	14 (48.3%)	15 (51.7%)	29 (29%)	
≥ 46	21 (53.8%)	18 (46.2%)	39 (39%)	
Total	56 (56%)	44 (44%)	100 (100%)	

Table (4.4): Shows the distribution of ESBLs producer and non-ESBLs producer *E. coli* isolates among inpatients and outpatients departments.

Department	ESBLs		Total NO - (%)	P. value
	Positive NO - (%)	Negative NO - (%)		
Inpatient	51 (64.6%)	28 (35.4%)	79 (79%)	0.001
Outpatient	5 (23.8%)	16 (76.2%)	21 (21%)	
Total	56 (56%)	44 (44%)	100 (100%)	

Table (4.5): Antimicrobial susceptibility result for ESBLs and non-ESBLs producer of uropathogenic *Escherichia coli.*

Antibiotic	S (%)	I (%)	Resistance %			P.value
			ESBL (56) NO – (%)	NON ESBL (44) NO- (%)	Total resistance	
IPM	100	0	0	0	0	0
CAZ	23	5	55 (98.2%)	17 (38.6%)	72	0.001
CTX	22	7	56 (100%)	15 (34.1%)	71	0.000
CRO	14	18	56 (100%)	12 (27.3%)	68	0.000
TZP	88	4	7 (12.5%)	1 (2.3%)	8	0.027
TE	18	23	40 (71.4%)	19 (43.2%)	59	0.014
SXT	20	12	44 (78.6%)	24 (54.5%)	68	0.007
CIP	36	2	48 (85.7%)	14 (31.8%)	62	0.005
NOR	16	11	50 (89.3%)	23 (52.3%)	73	0.000
AK	63	5	27 (48.2%)	5 (11.4%)	32	0.000

S: Sensitive, I: Intermediate.

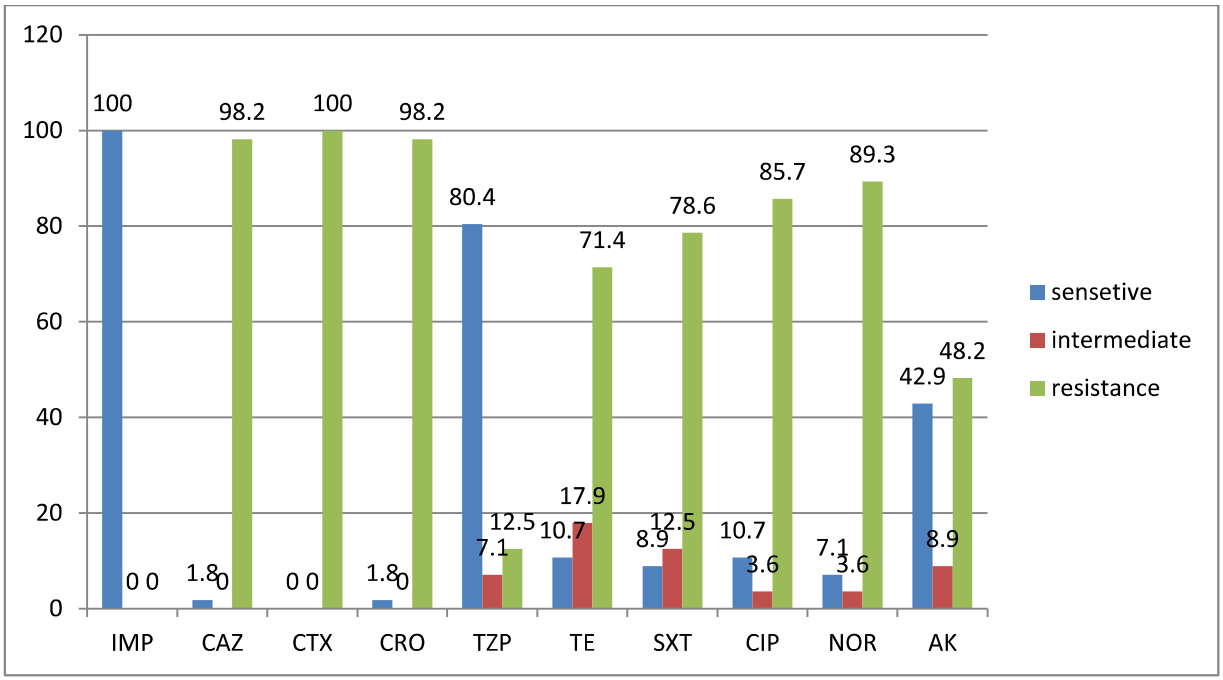

Figure 4.1: The susceptibly pattern of 56 ESBL producing *E. coli* isolates to 10 antimicrobial agents.

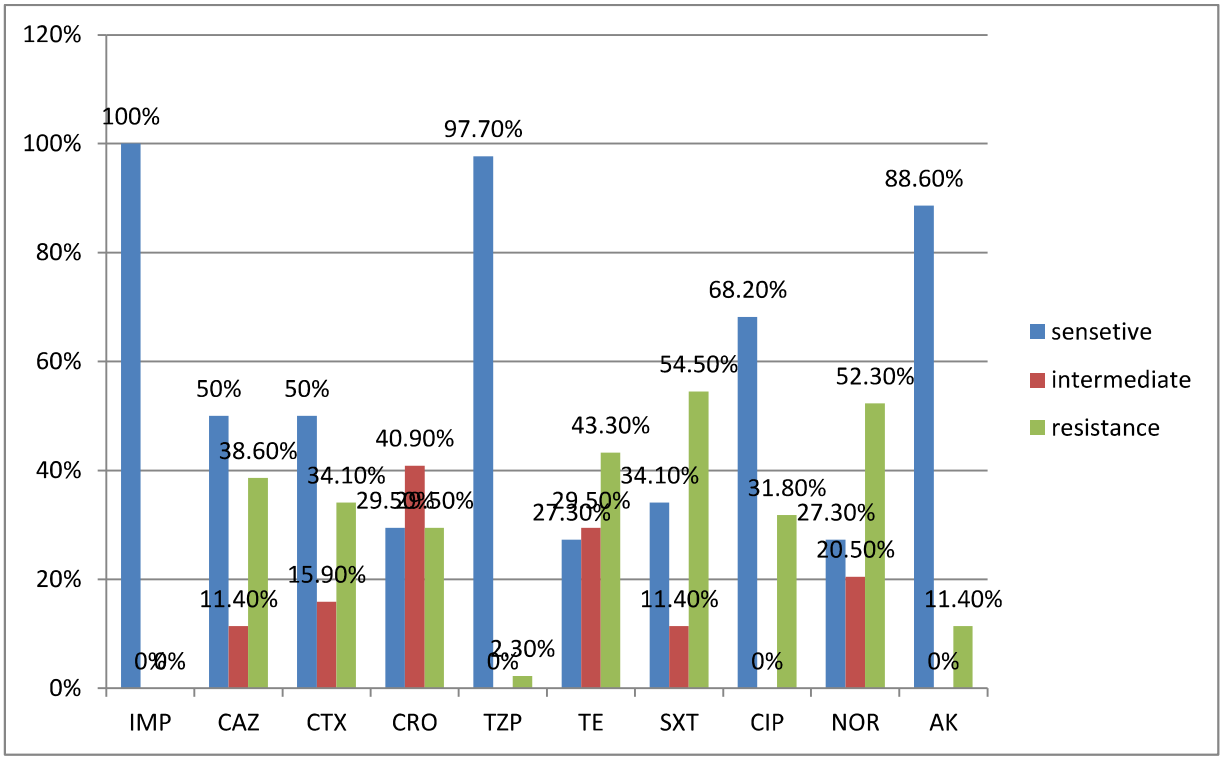

Figure 4.2: The susceptibly pattern of 44 non ESBL producing *E. coli* isolates to 10 antimicrobial agents.

Chapter Five

Discussion
Conclusion
Recommendations

5.1. Discussion

Dissemination of ESBL-producing bacteria could be attributed to the presence of multiple risk factors such as inappropriate use of broad-spectrum antimicrobials, inappropriate prescription, and long duration of hospital stay and transfer of ESBL genes by transposable elements such as plasmid and integron in health care settings (Ahmed *et al*., 2013).

The present study showed that out of 100 tested isolates *E.coli* 56 (56%) were ESBL positive. However this finding was higher than other studies which carried out in different region of the world such as (Martinez *et al*., 2012) in Colombia, (Harada *et al* ., 2013) in Japan (and (Hassan *et al*., 2014) in Saudi Arabia. These studies reported the ESBL positive as 11.7%, 20.4% and 35.8% respectively. In regarding to Sudan these Ibrahim *et al*. (2013) and Almugadam *et al*. (2016) reveal the following frequency 30.2% and 35% respectively. Moreover this study reveals ESBL positive lower than these Sharma *et al*. (2013) in Uganda, Bali *et al*. (2010) in Turkey, Fernandes *et al*. (2014) in Portugal and Salem *et al*. (2010) in Egypt. That reported the ESBL positive as 62%, 84%, 67.9% and 87% respectively. Nevertheless, the current study findings are similar to studies done by (Ejaz *et al*., 2011), (Goudarzi *et al*., 2014), (Sharma *et al*., 2013) who finding ESBL positive were 57.4%, 55.5% and 57.2% respectively. These findings indicate that the prevalence of *E.coli* producing-ESBL varies worldwide and these differences could be attributed to type and volume of samples, duration of study and drug regimens in different geographical regions.

In this study gender is highly affected by isolated *E. coli producing* ESBL, through that, females demonstrate a (75%) compare to (25%) for males, with P.value (0.005). And this in agreement with findings of study done by (Nwosu *et al*., 2014) that reported ESBLs producers were higher in females (72.8%%) than males (27.2%), and disagreement with study done by (Vidhya *et al*., 2013) who

reported no significant differences between male (47.22%) and female (52.77%) in ESBL producer isolates.

In the present study there were no significant differences between age group in ESBL producing *E. coli* isolates. But the most infected age group with ESBL-producing E. coli was ≥ 46 years age group 53.8% (21/39) followed by ≤ 16 years age group 62.5% (20/32) and 17-45 years 48.3% (14/29).This finding agreement with study done by (Ibrahim *et al* ., 2013) who found no significant differences between ESBL-producing isolates of adults(31%) and (27%)children. But a previous study (Moyo *et al*., 2010) reported significantly higher ESBL production in isolates from children rather than adults.

In this study there was a significant differences in ESBLs producing *E. coli* isolates from inpatients department (89.3%) and outpatients department (10.7%), with P.value (0.05).This study agree with other studies revealed significant high rate inpatient department infected with ESBL producing *E. coli*. (Mahrgan & Rahbar, 2008). While Coqu *et al*. (2008) and Khanfar *et al*. (2009) reported most commonly community acquired infections.

This study showed that all ESBL-producing *E. coli* isolates were significantly more resistant to ceftazidime, cefotaxime, ceftriaxone, piperacillin/tazobactum, Tetracycline, Co-trimixazol, Ciprofloxacine norfloxacine and amikacin compared to non-ESBL producing isolates with ($p<0.05$) as shown in Table(4.5) . These finding agrees with study of (Ibrahim *et al*., 2013) except the amikacin. The high resistances of ESBL-producers isolates were to cefotaxime (100%), ceftriaxone (100%), ceftazidime (98.2%) and norfloxacine (89.3%), while the high resistances of non ESBL-producers isolates were to co-trimixazol (54.5%), norfloxacine (52.3%) and tetracycline (43.2%). However the maximum sensitivity of both ESBL-producers and non-producers isolates were seen for imipenem (100% vs.100%), followed by piperacillin/tazobactum (80.4% - 97.7%) and amikacin

(42.9% - 88.6%)). This finding is in agreement with study done by (Goudarzi *et al*., 2014) who reported the resistance of ESBL-producing isolates were to cefotaxime (98.7. %), ceftriaxone (97.3%), ceftazidime (81.3%). The lowest rates of resistance in ESBL-producing isolates were observed for imipenem (2.7%) and amikacin (14.7%).

A possible reason of high resistance in this study might be contributed to the presence of ESBL in these strains. Overall, this is an alarm for clinicians that consumption and prescription of these antibiotics must be changed.

5.2. Conclusion

The information from this study revealed that there was a high prevalence of extended spectrum β-lactamases (ESBLs)-producing *E. coli* infection among urinary tract infected patients. Females had a higher rate of ESBL producing *E. coli isolates* compared to isolates from males, this differences was a significant in this study. However, the age group shows no significant differences in ESBL producing *E. coli* isolates. a significant differences in the ESBL producing *E. coli* isolates regarding department distribution showing that inpatients department had higher rate than outpatients department. Furthermore, the ESBL producing *E. coli* isolates were more resistance to antimicrobial agent than non ESBL producer but the Imepemen remain the most effective agent for ESBL producing *E. coli* causing UTIs.

5.3. Recommendations

1. Genotype characterization and molecular techniques are required to explore genes responsible for production of extended spectrum β-lactamases (ESBLs) *E.coli* infection.
2. Further studies in ESBL producing bacteria in urinary tract infection and other populations with other side infection.
3. Treatment of extended spectrum β-lactamases (ESBLs) *E. coli* infection with IPM is recommended.

References

Appendices

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Appendices

Appendix NO: 1 approval

بسم الله الرحمن الرحيم

جامعـــــة شنـــــدي

كلية الدراسات العليا والبحث العلمي

مركز الخرطوم

النمرة : ج ش/ك د ع /م خا 2018/4/23م

إلى من يهمهم الأمر بمستشفى سوبا الجامعي

المحترمين

السلام عليكم ورحمة الله وبركاته

الموضوع : الطالب/محمد حسين حسين

بالإشارة إلى الموضوع أعلاه نفيدكم بأن الطالب المذكور من ضمن طلاب الكلية بالفصل الدراسي الرابع – ببرنامج ماجستير علوم المختبرات الطبية –الاحياء الدقيقة ، لدية مطلوبات لاكمال بحثه التكميلى بعنوان :

Prevalence of extended spectrum beta lactamase producing Escherichia coli in urinary tract infection at Khartoum state.

نرجو منكم تيسير مهمته البحثية .

ولكم فائق الشكر والتقدير،،،،

الصادق أحمد عبدالقادر

مسجل المركز

Appendix NO 2

Colure plats

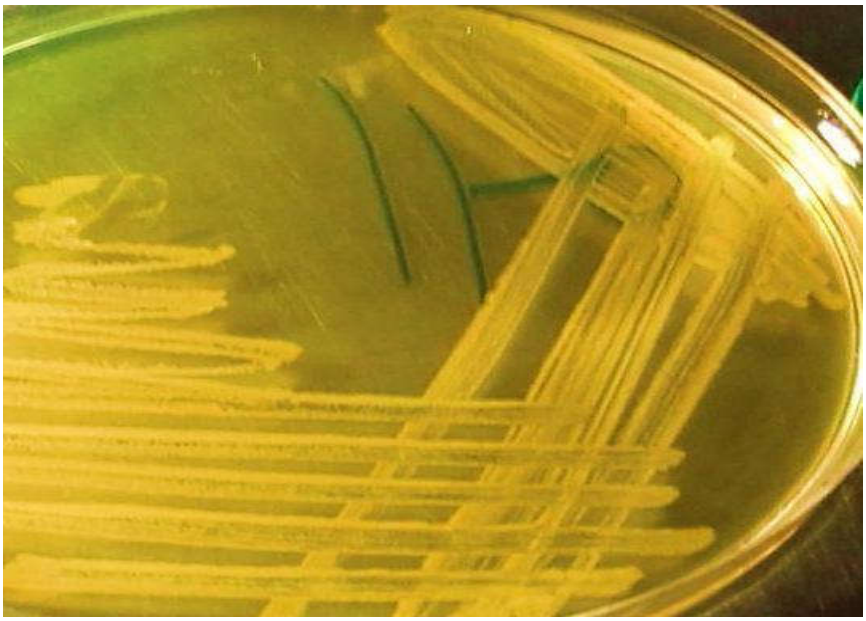

Plate 1: *E. coli on* CLED media showing yellow color indicating lactose fermentation.

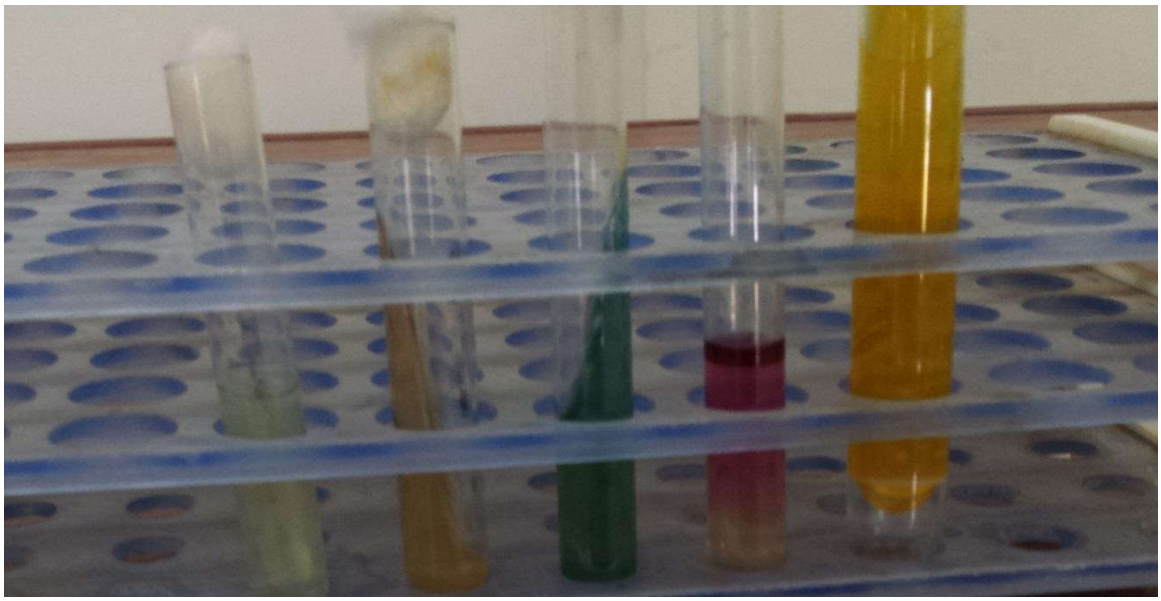

Plate 2: *E. coli* biochemical set/from right to left, KIA: slope yellow, butt yellow ,Gas positive, H_2S negative; Indole test, positive; Citrate test, negative; Urease test, negative; Motility test, motile.

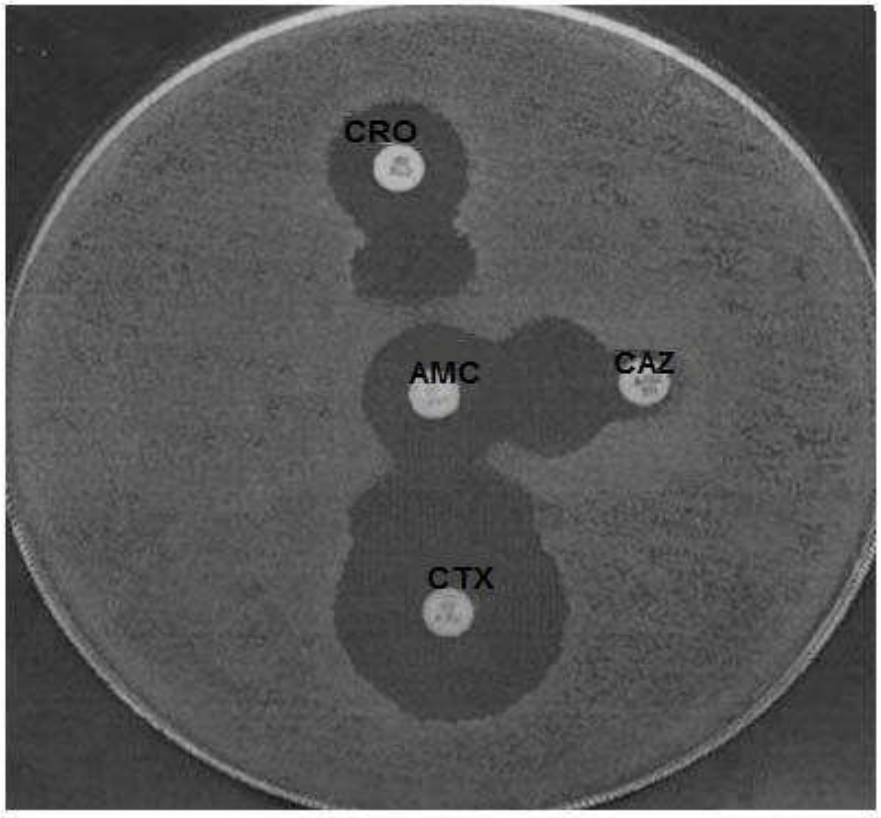

Plate 3: Double disk synergy of ESBL producers *E. coli on* Muller Hinton Agar after overnight incubation.

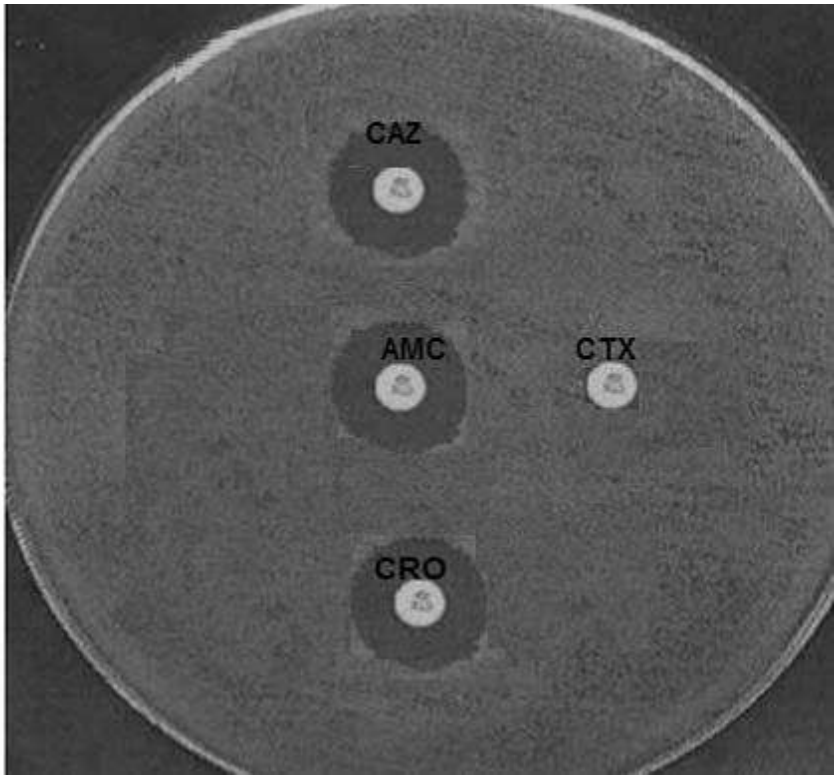

Plate 4: Negative ESBL producers *E. coli on* Muller Hinton Agar after overnight incubation.

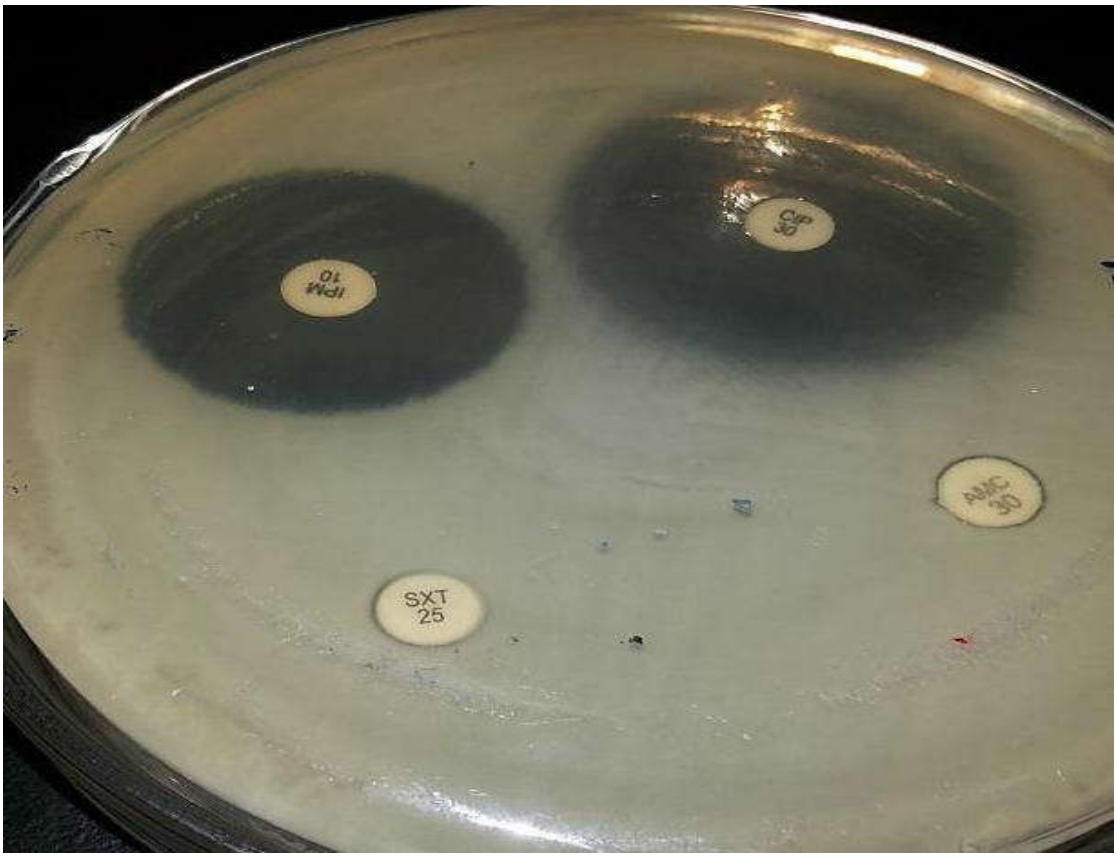

Plate 5: Resistance to antibiotics by *E. coli.* The figure shows sensitivity to Imepenem and Ciprofloxacin and resistance to Amoxicillin and Co-trimoxazole.

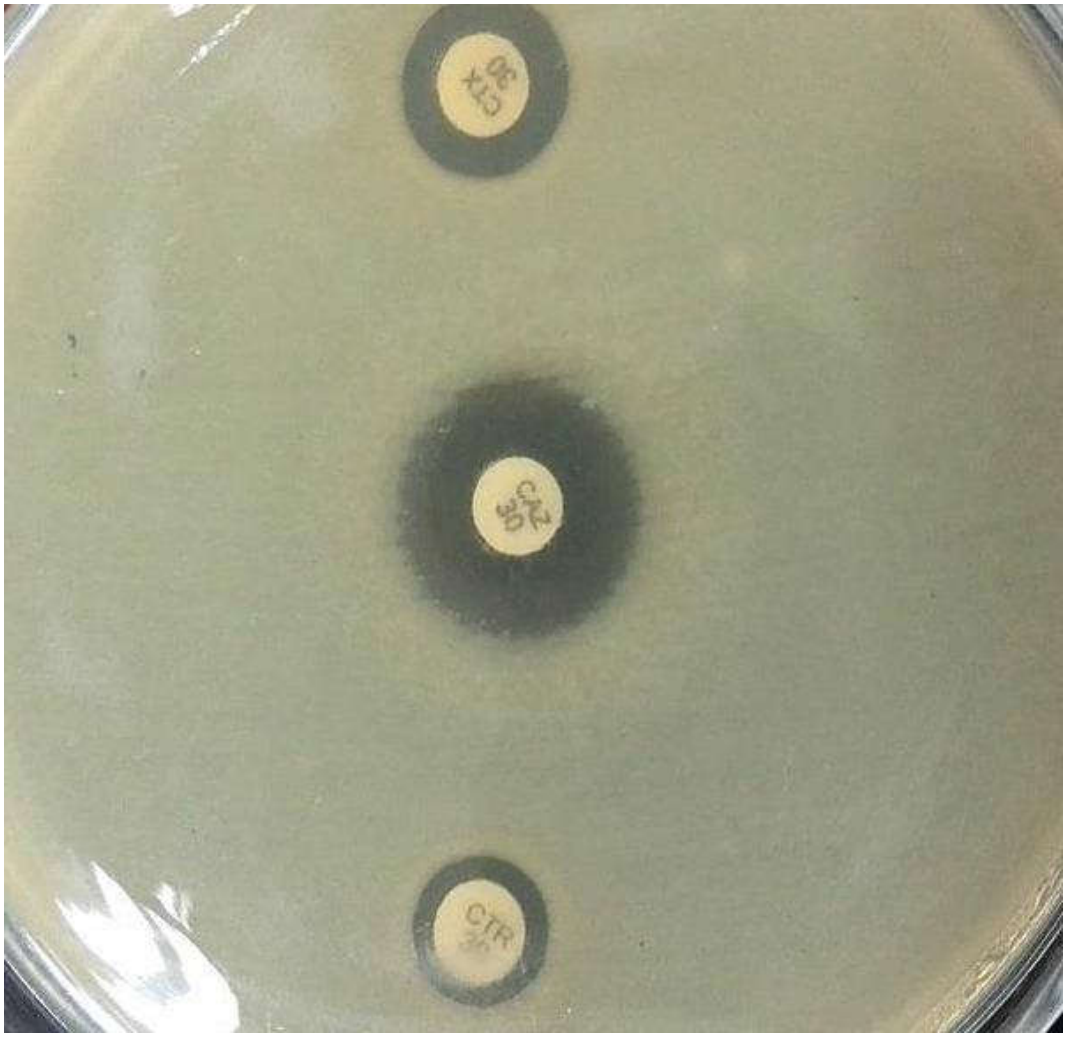

Plate 6: Resistance to third generation cephalosporins (ceftazidime, cefotaxime and ceftrixone) by *E. coli.*